Phytochemical Properties & Antimicrobial Potential of Selected Medicinal Plants for Diabetes

(An Study of Kashmir Valley, J&K, India)

A Synopsis

Presented for Awarding the

Doctor of Philosophy Degree in Biochemistry

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2020

INTRODUCTION :

Pathogenic microbes have always posed serious threats to the health of humans and other animals. Microbes such as bacteria, viruses, fungi or protozoans are the causative agents of numerous infectious diseases like Tuberculosis, AIDS, Syphilis, candidiasis, aspergillosis, amoebiasis, poliomyelitis, etc. In fact infective diseases are the second leading cause of death worldwide (WHO, 2002a). Microbes existed on earth since million years ago, being one of the oldest creature on this planet. The conflict between man and microbes began since his appearance on earth. Man used the antimicrobial drugs against microbes since times immemorial.

Medicinal plants were the first weapons that the man used against pathogenic microbes. However with the discovery of antibiotics in 20^{th} century, scientific community begain to synthesize synthetic or semisynthetic antimicrobial drugs but ironically, the misuse of antibiotics by human, the employment of antibiotics in veterinary practices and the growing presence of antibiotics in water, soil and food have contributed to the problem of antibiotic resistance (Moshirfar *et al*., 2006).

Antimicrobial resistance is a serious global challenge and could endanger the lives of future generations. After more than 50 years of widespread use of so called “miracle drugs”, synthetic antibiotics are no longer as effective as they once used to be. Virtually most of the bacterial infections throughout the world are becoming resistant to antibiotics (Johnson, 2006). And even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs that are used as therapeutic agents (Nascimento *et al*., 2000).

As resistance to antibiotics becomes more common there is greater need for alternative treatments. Antimicrobial resistance (AMR) can occur in both humans and other animals. It can spread from one infected person to other person or from animals to humans and vice versa. Out Of the two million people who acquire bacterial infections in U.S. hospitals each year, 70% of cases now involve strains that are resistant to atleast one drug. In U.K., Methicillin resistant *staphylococcus aureus* (MRSA), which was at low level a decade ago, has now increased to about 50% of all *Staphylococcus aureus* isolates (Bader, 2013).

Antimicrobial resistance occurs due to the excessive use of antimicrobials itself. Since 37 years ago, no new classes of antibiotics were discovered and all

antibiotics that entered the markets during this period were modification of existing molecules (Coates *et al.*, 2002). It seems that there is no obvious hope to new antibiotic generations in the near future unless and until rigorous efforts are made.

The plant kingdom is a treasure house of potential drugs. It is estimated that there are about 2.0 lakh to 5.0 lakh species of plants on earth. Among them only a negligible percentage has been explored for phytochemicals and medicinal properties. In fact only less than 1% of some 250,000 higher plants have been screened for their phytochemistry or pharmacology (Petlevski et al., 2001). Medicinal plants have been a part of traditional healthcare on all the continents of the world for thousands of years. There is evidence that Neanderthals living 60,000 years ago in present day Iraq used plants such as hollyhock (*Alcea rosea*) (Stockwell C, 1988 and Thomson WAR, 1978) for medicinal purpose.

These plants are still used widely in ethnomedicine around the world. According to a report of World Health Organisation (WHO), more than 80% of world's population depends on traditional medicine for their primary health care needs (Umamaheshwari *et al*., 2008). In India more than 7000 medicinal plants are known. Many medicinal plants find their application in pharmaceutical, cosmetical agricultural and food industry.

Ayurvedic system of medicine is also based on the use of medicinal plants for the cure of diseases. In the recent years, there has been an increasing conciousness about the grandedness of medicinal plants. Medicinal plants have multiple therapeutic functions such as anti-oxidant, anti-inflammatory, anticancerous as well as anti-microbial activities. They possess such properties due to the presence of various phytochemicals in their parts like root, stem, leaves, bark, fruit and seeds which produce a definite curing physiological response.

Medicinal plants are rich in a numerous variety of secondary metabolites of antimicrobial properties such as saponines, tannins, alkaloids, alkenyl phenols, glycoalkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phorbol esters (Tiwari and Singh, 2004; Lewis and Ausubel, 2006). Current global drug development program may not be able to provide new effective antibiotics in 10 to 20 years (Boucher *et al*, 2009).

However, medicinal plants are expected to be a better candidate for the development of future antimicrobials. Some representatives of the pharmaceutical industry are aware of this potential and have introduced screening programs for medicinal plants, particularly from tropical regions (Hostettmann *et al*., 2000). Phytoconstituents with antimicrobial potential are the emerging alternatives for the control of pathogenic microorganisms.

REVIEW OF LITERATURE :

The following Research studies are observed for better Research Work.

- Medicinal Plants have been traditionally used for therapeutic purposes all over the the world since antiquity to date. Hippocrates (460–377 BC), one of the ancient authors listed about 400 different plant species for medicinal purposes. Natural products have been an integral part of ancient medicinal systems, e.g. **Chinese, Ayurvedic and Egyptian (Sarker and Nahar, 2007).**
- A single plant may be used for the treatment of various disease conditions in different ethnic communities worldwide. Several ailments including, asthma, fever, esophageal cancer, constipation and hypertension have been treated with traditionally used medicinal plants **(Cousins and Huffman, 2002; Saganuwan and Saganuwan, 2010).**
- The plants are applied in different forms or preparations such as concoctions of different plant mixtures, poultices, infusions as teas or tinctures or as component mixtures in porridges and soups administered in different ways including oral, nasal(smoking, snoffing or steaming), topical (lotions, oils or creams), bathing or rectal (anemeas).Different plant parts and components

(roots, leaves, stem barks, flowers or their combinations, essential oils) have been employed for the treatment of various infectious pathologies of respiratory system, urinary tract, gastrointestinal and biliary systems, as well as on the skin **(Gabriela *et al*., 2001; R´ıos and Recio, 2005; Adekunle and Adekunle, 2009).**

- Medicinal plants possess pharmacological activities due to the presence of secondary metabolites in them which are comparatively smaller in size as compared to the primary molecules such as proteins, carbohydrates and lipids. These natural products provide clues to develop new structural types of antimicrobial and antifungal chemicals that are relatively safe to humans **(Kalimuthu *et al.*, 2010).**
- **Sharvani *et al* (2015)** studied the antimicrobial activity of six species of *Elaeocarpus* against certain bacterial and fungal strains and found that all the species were potent against the tested microbes. **Saba Irshad et *al* (2012)** reported the antibacterial potential of three medicinal plants namely *saussurea lappa*, *Acacia nilotica* and *Ricinus communisa* gainst four bacterial strains namely *Lactobacillus subtilis*, *Bacillus thureogenesis*, *corney bacterium* and *Escherichia coli*.

- **Nisar *et al* (2012)** evaluated the antimicrobial potential of twenty plants against three bacterial (*E.coli, Staplylococcus aureus, salmonella typhii*) and two fungal strains (*Alternaria alternate, Fusarium oxysporium*). The results revealed that *Albizia lebbeck was* most potent against bacterial strains while as *withania coagulans* and *sida spinosa* showed potent antifungal activity **Kumara et al (2012)** confirmed the antibacterial and antifungal potential of bougainvillea spectabilis flower extracts.
- Many studies have been carried in Kashmir valley involving the evaluation of antimicrobial potential of medicinal plants. **Irshad et al (2012)** evaluated the antibacterial potential of Hypercum *perforatum*. The plant was found potent against *Staphylococcus epidermidis, B. subtilis, Proteus vulgaris, S.aureus, Salmonella typhi* and *E.coli*.

OBJECTIVES OF RESEARCH :

The following Objectives are based on my Research Study:

1. Phytochemical Analysis: To examine Preliminary phytochemical analysis of the plant powder and different extracts will be carried out by using various standard methods.

2. Anti bacterial activity: To examine test extracts for antibacterial potential against certain pathogens by various approved methods.
3. Antifungal activity: To examine test extracts for antifungal potential againt certain pathogens by various approved methods.

HYPOTHESIS :

I made the following Hypothesis for testing and to examine during my Research Work:

1. With the advent of antimicrobial resistance and with with no new antibiotics, plant extracts are becoming increasing a new area of research with their phytochemical, antibacterial and antifungal properties.
2. Kashmir valley being the rich source of medicinal plants has to be a source of various principle compounds which could be harnessed for phytochemical and antimicrobial properties.
3. There is need to explore more from the natural rersources to find an acceptable option in the form of a potion or purified drug.
4. A plant extract, because of its very interesting cocktail of natural origin can act on different targets amid low toxicity and inexpensive status and they

have been used as a preferred scouge for treatment of many diseases over the years.

AREA OF RESEARCH :

My Research Work area is The Valley of Kashmir. The *valley of Kashmir* is situated in the northern fringe of the Indian sub continent between 33°22' and 34°50 N latitudes and 73°55' 73°33 E longitudes covering an area of about 16000 square kilometers. Kashmir Himalayas harbours a rich diversity of medicinal plants chiefly owing to its topographic variations spanning from the valley floor through terraced tablelands (Karewas) and dense forests upto alpine peaks.

The valley is also known as the biomass state of India due its richness in aromatic and medicinal plants **(Ganie *et al.*, 2013)**. More than 50% of plant species described in british pharmacopoeia are reported to grow in Kashmir valley and it is established that nearly 570 plant species of medicinal importance grow here. **(Mudasir A., 2014)**. **Kachroo and Nahvi (1976)** have listed a total number of 103 medicinal plant species used in early Kashmir. **Koul (1997)** listed a total of 111 species of medicinal plants from Kashmir and Ladakh while **Dar *et al.* (2002)** has listed 450 plants of medicinal importancele. Also, **Guna (2006)**, has reported 220 medicinal plant species, belonging to 178 genera distributed over 77 families being used in Kashmir.

As a matter of fact, the medicinal flora of Kashmir valley has yet not received the attention that it deserves. **(Koul, 1997)**. Therefore the present study

has been carried out to evaluate the antimicrobial potential of some medicinal plants of Kashmir valley against various bacterial and fungal strains. The study could serve as a beginning step in the development of future antimicrobials.

RESEARCH DESIGN :

My Research Design is based on my Research Topic. There are three types of research I will use in my Research Work.

1. Exploratory Research
2. Descriptive Research
3. Experimental Research

Phytochemical screening **:** Qualitative phytochemical screening of both the aqueous and methanolic extracts will be carried out to know the nature of phytochemicals present in them. Flavanoids are detected by lead acetate test while the rest of phytochemicals will be detected by the methods described earlier [Harborne, 199].

Test for steroids: To 0.5 ml of solvent extract, 2ml of acetic acid is added and then 2ml of concentrated sulphuric acid is added. Appearance of Blue or green colour or

a mixture of these two shades is regarded as positive for the presence of steroidal compounds.

Test for tannins:To 5ml of solvent extract, two drops of 5% $Fecl_3$ is added. Production of greenish precipitate indicated the presence of tannins.

Test for terpenoids :To 5 ml of solvent extract, 2ml of chloroform is added and then 3ml of concentrated sulphuric acid is added carefully. Appearance of reddish brown colouration of the interface will be regarded as positive for the presence of terpenoids.

Test for flavonoids :To 2 ml of solvent extract, a few drops of lead acetate solution is added. Formation of yellow coloured precipitate will be regarded as positive for the presence of flavanoids.

Test for alkaloids :To 2ml of solvent extract, a little amount of picric acid solution is added. Formation of orange colour indicated the presence of alkaloids.

Test for saponins :About 1 ml of solvent extract is introduced into a tube containing 1ml of distilled water and the mixture is vigorously shaken for 2 minutes. Formation of froth indicated the presence of saponins.

Test for anthraquinones : 2ml of solvent extract is added to 10 ml of benzene, and then 0.5ml of ammonia solution is added. The mixture is shaken well. Voilet colour in the layer phase indicated the presence of anthraquinones.

Test for phenols : To 2 ml of solvent extract, 2ml of ferric chloride solution is added. Formation of deep bluish green solution indicates the presence of phenols.

Test for cardiac glycosides : To 2ml of solvent extract, 2 ml of glacial acetic acid containing 1 drop of ferric chloride is added. Then 2ml of concentrated sulphuric acid (H_2SO_4) is added under layered. Formation of brown ring at the interface indicated the presence of cardiac glycoside.

Test for cardenolides : To 1 ml of solvent extract, 2ml of benzene is added. Appearance of turbid brown colour indicated the presence of cardenolides.

Test for phlobtannins : To 1 ml of solvent extract, few drops of 1% hydrochloric acid is added and the mixture is boiled. Formation of red precipitate indicated the presence of phlobtannins.

Test for volatile oils : To 2 ml of solvent extract, 0.1ml of sodium hydroxide and then small amount of diluted hydrochloric acid is added and shaken well. Formation of white precipitate indicated the presence of volatile oils.

Test micro-organisms : For antibacterial assay, a total of six bacterial strains including two Gram positive bacterias namely *Staphylococcus aureus* (MTCC-2940), *Bacillus subtilis* (MTCC-441) *and four* Gram negative bacterias namely *Klebsiella pneumoniae* (MTCC-139), *Proteus vulgaris* (MTCC-426), *Pseudomonas aeruginosa* (MTCC-424) and *Escherichia coli* (MTCC-739) will be used. Similarly for antifungal assay, four fungal strains including *Candida albicans* (MTCC-227), *Saccharomyces cerevisiae* (MTCC-170), *Aspergillus fumigatus* (MTCC-1811) and *Penicillium chrysogenum (*MTCC-947) will be used. All Bacterial and fungal strains will be obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India.

Negative and positive controls : Gentamycin (discs) and Nystatin (powder) will be obtained from EOS Laboratories, India. Gentamycin and Nystatin will serve as positive controls for antibacterial and antifungal assay respectively. 10% Dimethylsulfoxide (Himedia) alone will served as negative control in both the assays.

For determination of antibacterial and antifungal assay ,preparation and standardization of bacterial and fungal inoculums will be done. Antibacterial and antifungal assays of the aqueous and methanolic extracts will be carried out by using agar well diffusion method.

Calculation of percentage relative inhibition zone diameter (%RIZD)

Percentage relative inhibition zone diameter (% RIZD) will be calculated by the following formula (Hussain et al., 2014):

$$\%\mathbf{RIZD} = \frac{\mathbf{IZD\ of\ extract - IZD\ of\ negative\ control}}{\mathbf{IZD\ of\ positive\ control}} \times \mathbf{100}$$

Where, IZD= Inhibition zone diameter (measured in millimeter)

(Note: % RIZD was calculated at the concentration of 100 mg/ml).

SAMPLING METHOD & SLECTION OF SAMPLES :

I will used purposive sampling method in selection of samples for my research work. Will be collected from different areas of Kashmir Valley, India. All plants are identified in the Centre of Plant Taxonomy (COPT), Department of Botany, University of Kashmir, India. Specimen of each plant is retained in the KASH herbarium of COPT under a specific voucher specimen number. Whole plant samples will be thoroughly washed under running tap water to get rid of all the debris and soil and then cut into small pieces and shade dried at room temperature. The plant material will be ground into coarse powder with the help of electric grinder and extracted successively using methanol and water respectively, by Soxhlet extraction. The extracts so obtained will be concentrated by evaporating excessive solvents in a water bath and then stored in a refrigerator at 4^{o}c.

I will select 21 following medicinal plants -

1. Datura stromonium
2. Coriandum sativum.
3. Mentha arvensis
4. Solanum Nigrum
5. Portulaca Oleraceae.
6. Fragaria Nubicola

7. Amaranthus Caudatus.
8. Adiantum capillus.
9. Prunella Vulgaris.
10. Strobilianthes Urticifoliawall.ex kuntze.
11. Rumex Dendatus.
12. Hedera Nepalensis.
13. Trigoneella Emodi.
14. Mariubium Vulgare.
15. Pseudophegopteris Levingei.
16 Malva Sylvestris.
17. Taraxacum Officinale.
18. Viola Odorata.
19. Artemisia Absinthium.
20. Urtica Dioica.
21. Rheum Spiciformis.

CHAPTERIZATION :

My Research Work will be divided into following Chapters -

Chapter-1 : Introduction of Research Study.

Chapter-2 : Review of Literature.

Chapter-3 : Experimental Procedure & Method.

Chapter-4 : Results & Analytical Presentation

Chapter-5 : Summary, Conclusion & Suggestions

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